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Comparison of the local pulmonary distribution of nanoparticles administered intratracheally to rats via gavage needle or microsprayer delivery devices

Guihua Zhang[a], Naohide Shinohara[a]*, Yutaka Oshima[b], Toshio Kobayashi[b], Nobuya Imatanaka[c], Kenji Kawaguchi[d] and Masashi Gamo[a]

ABSTRACT: Intratracheal administration methods are used to conduct toxicological assessments of inhaled nanoparticles (NPs), and gavage needles or microsprayers are common intratracheal delivery devices. The NP suspension is delivered in a liquid state via gavage needle and as a liquid aerosol via microsprayer. The differences in local pulmonary NP distribution (called the microdistribution) arising from the different states of the NP suspension cause differential pulmonary responses; however, this has yet to be investigated. Herein, using microbeam X-ray fluorescence microscopy, we quantitatively evaluated the TiO_2 pulmonary microdistribution (per mesh: 100 μm × 100 μm) in lung sections from rats administered an intratracheal dose of TiO_2 NPs (6 mg kg^{-1}) via gavage needle or microsprayer. The results revealed that: (i) using a microsprayer appears to reduce the variations in TiO_2 content (ng mesh^{-1}) among rats (e.g., coefficients of variation, $n = 3$, microsprayer vs gavage needle: 13% vs 30%, for the entire lungs); (ii) TiO_2 appears to be deposited less in the right middle lobes than in the rest of the lung lobes, irrespective of the chosen intratracheal delivery device; and (iii) similar TiO_2 contents (ng mesh^{-1}) and frequencies are deposited in the lung lobes of rats administered TiO_2 NPs via gavage needle or microsprayer. This suggests that the physical state of the administered NP suspension does not markedly alter TiO_2 pulmonary microdistribution. The results of this investigation are important for the standardization of intratracheal administration methods. Copyright © 2016 John Wiley & Sons, Ltd.

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Keywords: intratracheal administration; delivery device; gavage needle; microsprayer; pulmonary microdistribution; TiO_2; XRF

Introduction

As applications for nanoparticles (NPs) continue to spread across fields, concerns about their safety have been raised. Intratracheal administration methods have been used for the toxicological assessment of inhaled NPs (Driscoll *et al.*, 2000; Morimoto *et al.*, 2016). These methods include gavage needles (Jacobsen *et al.*, 2015; Yoshiura *et al.*, 2015) and microsprayers (Shinohara *et al.*, 2014; Tada *et al.*, 2013). Gavage needles deliver the NP suspension in a liquid form. In contrast, via microsprayer, the NP suspension is sprayed as a liquid aerosol; this appears to mimic the conditions of inhalation exposure studies more accurately. To determine NP toxicity, microscopic histopathological examinations may be performed on lung tissue (Jacobsen *et al.*, 2015; Yoshiura *et al.*, 2015). It is anticipated that differences in the local NP pulmonary microdistribution arising from the different administration methods, may lead to differential pulmonary responses to the administered NPs. However, few studies have been conducted comparing two different intratracheal delivery devices and the differences in NP pulmonary microdistribution.

Microbeam X-ray fluorescence (XRF) analysis has been previously applied for the evaluation of NP microdistribution in biological samples (Wang *et al.*, 2007, 2008). To quantify the NP microdistribution, we have successfully developed a new set of titanium reference samples to evaluate quantitatively the TiO_2 pulmonary microdistribution (per mesh: 100 μm × 100 μm) in rats having been administered a suspension of TiO_2 NP using a microsprayer (Zhang *et al.*, 2015, 2016).

In the present study, we aimed to investigate the effect of NP suspension physical state (liquid vs aerosol) on the pulmonary microdistribution of NPs. To this end, we administered a single dose of TiO_2 NPs to rats (6 mg kg^{-1} body weight), using either gavage needle or microsprayer, and quantitatively evaluated TiO_2 pulmonary microdistribution in lung lobe sections using microbeam XRF microscopy.

*Correspondence to: Naohide Shinohara, National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Ibaraki 305-8569, Japan.
E-mail: n-shinohara@aist.go.jp

[a]National Institute of Advanced Industrial Science and Technology (AIST), Ibaraki 305-8569, Japan

[b]Chemicals Evaluation and Research Institute (CERI), Hita, Oita, Japan

[c]Chemicals Evaluation and Research Institute (CERI), Tokyo 112-0004, Japan

[d]National Institute of Advanced Industrial Science and Technology (AIST), Ibaraki 305-8565, Japan

Materials and methods

Intratracheal delivery devices

A gavage needle (0.9 mm × 70 mm; Natsume Seisakusho Co., Ltd., Tokyo, Japan) and a microsprayer (MicroSprayer® Aerosolizer, 0.032 mm (tip) × 75 mm; Model IA-1B, Penn-Century, Inc., Wyndmoor, PA, USA) were used for the intratracheal administration of the TiO_2 NP suspension.

Preparation and characterization of the TiO_2 suspension

In the current study, AEROSIL® P25 (>99.5% purity; Nippon Aerosil Co., Ltd, Tokyo, Japan) was used to prepare the suspension of TiO_2 NPs. AEROSIL® P25 has a spherical primary particle size of 21 nm, with a mixture of anatase and rutile phases in a 80:20 ratio, as per the company's technical information. The primary particle size of P25 was found to be 24 ± 7.9 nm (mean ± SD) using transmission electron microscopy (JEM-2010; Japan Electro Optical Laboratory Ltd., Tokyo, Japan), and the specific surface area was 59 $m^2 g^{-1}$. The details regarding the preparation of the P25 suspension are described in our previous study (Zhang *et al.*, 2015). The concentration of the P25 suspension (5.86 mg ml^{-1}) was measured using a weight scale after drying the suspension in a thermostatic chamber (ON-300S; AS ONE Co., Japan). The number-based average particle size of P25 suspension was determined to be 86 nm using DLS (Zetasizernano-ZS; Malvern Instruments Ltd., UK). The number-based agglomerate size of the P25 NPs in the suspension was similar when passing through the gavage needle or microsprayer (Supplemental Fig. S1).

Experimental procedure

The animals were cared for in accordance with our laboratory's guidelines for animal experiments, which comply with the regulations of the: Ministry of the Environment; Ministry of Health, Labour and Welfare; Ministry of Agriculture, Forestry and Fisheries; and Ministry of Education, Culture, Sports, Science and Technology. The study was approved by the Animal Care and Use Committee of the Chemicals Evaluation and Research Institute and the Institutional Animal Care and Use Committee of the National Institute of Advanced Industrial Science and Technology.

Male F344/DuCrlCrlj rats (SPF) were obtained from Charles River Laboratories Japan, Inc. (Kanagawa, Japan). The animals were quarantined and acclimated for 8 days. All animals were housed individually in stainless-steel wire hanging cages (170 mm width × 294 mm depth × 176 mm height) under controlled environmental conditions in the barrier controlled animal rooms (temperature of 23 ± 2 °C and relative humidity of 55% ± 15% with 15–17 air changes per hour). Fluorescent lighting was controlled automatically to provide a 12 h light/dark cycle. All rats had free access to sterilized water and γ-irradiation sterilized commercial basal diet (CRF-1; Oriental Yeast Co., Ltd., Tokyo, Japan).

In this study, the total dosage of TiO_2 NPs was set at 6 mg kg^{-1} body weight. At 12 weeks of age, the rats were divided into two groups ($n = 3$ for each group) for the intratracheal administrations of TiO_2 NPs via either gavage needle or microsprayer. After inhalational anesthetization with 3.5% isoflurane gas (Forane; Abbott Japan Co., Ltd., Tokyo, Japan), the rats were administered the TiO_2 NP suspension (1 ml kg^{-1} body weight) intratracheally. During the administration, each rat was supported by a nylon band under its upper incisors and placed on a slanted board (at a 45° angle). The delivery device was inserted into the trachea at a depth of 6 cm from the angulus oris of the rat.

Twenty-four hours after intratracheal administration, the rats were euthanized under intraperitoneal pentobarbital anesthesia by exsanguination from the abdominal aorta, and the left and right cranial, middle, caudal and accessory pulmonary lobes were removed separately. Then, 3 μm thick sections of paraffin-embedded lung lobes were prepared. The details of this procedure can be found in our previous study (Zhang *et al.*, 2015).

Quantification of the TiO_2 pulmonary microdistribution using X-ray fluorescence microscopy

Using high-performance energy-dispersive XRF microscopy with a rhodium target X-ray tube (XGT-7200; Horiba Int., Kyoto, Japan), the spectral intensity of the Ti-Kα line (4.511 keV) was acquired for Ti quantification of selected rectangular sample areas. The analytical conditions for Ti were set as follows: beam size (spatial resolution), 100 μm; step size, 200 μm for lung sections from the TiO_2-treated rats; acquisition time, 60 s per point; excitation voltage, 50 kV; excitation current, 1 mA; full vacuum mode. The spectral intensity of Ti within a mesh with dimensions of 100 μm × 100 μm was measured for each analytical point.

In our previous study (Zhang *et al.*, 2015), we described the preparation and analysis of Ti reference samples (step size: 100 μm) to build the Ti calibration curve. The accuracy and validity of the developed quantitative method for the quantification of pulmonary TiO_2 deposition were also described.The net spectra intensity of each analytical point in the rat lung sections was calculated using the following equation:

$$I_{\text{net of each analytical point}}\,(\text{cps}) = I_{\text{n}} - I_{\text{bg}} \quad (1)$$

where $I_{\text{net of each analytical point}}$ is the Ti net spectral intensity of each analytical point; I_{n} is the Ti measured spectral intensity of each analytical point; and I_{bg} is the average Ti spectral intensity of the background (Zhang *et al.*, 2015). The content of TiO_2 in the mesh (100 μm × 100 μm) was quantified, using the Ti calibration curve obtained through the Ti reference samples. For each lung lobe, the entire right lung, or the entire lung, the average content of TiO_2 in the mesh was calculated by dividing the total content of TiO_2 with the number of meshes for each lung lobe, right lung or five lung lobe sections of each rat, respectively.

Statistical analysis

All statistical analyses were performed using SPSS software (IBM SPSS Statistics version 20; IBM Corp., Armonk, NY, USA). Student's *t*-test was used to compare (i) the content and detection rates of TiO_2, and (ii) frequency of TiO_2 detection falling within each of the six TiO_2 content ranges, in rats administered TiO_2 NPs via gavage needle and microsprayer. A one-way analysis of variance with Tukey's HSD test was used to compare the content and detection rates of TiO_2 among the lung lobe sections of rats administered TiO_2 NPs using the same delivery device. $P < 0.05$ indicated statistical significance.

Results

The content and detection rates of TiO_2 in different lung lobe sections 24 h after cessation of intratracheal administration of a

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single dose of TiO_2 NPs (6 mg kg^{-1} body weight) via gavage needle or microsprayer are presented in Table 1. The quantitative maps of the TiO_2 pulmonary microdistribution are shown in Fig. 1. The arrows on the lung sections represent the direction that the TiO_2 NP suspension entered the lungs (at the hilum).

In terms of the TiO_2 pulmonary microdistribution within the lungs of rats administered TiO_2 NPs via gavage needle or microsprayer, there were no significant differences in the content (including the mean, 95th percentile and maximum content) or detection rates of TiO_2 between each lung lobe, entire right or entire lungs (Table 1).

With regard to the TiO_2 pulmonary microdistribution among lung lobes, there was a similar deposition pattern in rats receiving TiO_2 NPs via gavage needle and microsprayer, i.e., relatively less deposition in the right middle lobes compared with the rest, although statistical significance for the content and detection rates of TiO_2 was not always observed.

The frequencies of TiO_2 detection within each of the six content ranges (including detection limit (DL) –0.1, 0.1–0.2, 0.2–0.3, 0.3–0.4, 0.4–0.5 and >0.5 ng $mesh^{-1}$) are plotted in Fig. 2. Each lobe, entire right lung and entire lungs showed similar frequencies of TiO_2 detection in rats administered TiO_2 NPs via gavage needle or microsprayer at almost every content range. Although there were significantly higher frequencies of TiO_2 detected in the range of 0.4–0.5 ng $mesh^{-1}$ in the entire lungs of rats administered TiO_2 NPs via a microsprayer than those via gavage needle, the values regarding the frequencies of TiO_2 detection were low at this content range (1.4% for microsprayer vs 0.60% for gavage needle, $P < 0.05$).

Furthermore, variations in the TiO_2 deposition in the lungs were larger for gavage needle than for microsprayer (coefficients of variation for the mean content of TiO_2 in a mesh, $n = 3$, gavage needle vs microsprayer: 36% vs 9.9% for the left, 99% vs 63% for the right cranial, 41% vs 30% for the right caudal, 49% vs 32%

Table 1. Data regarding the TiO_2 pulmonary quantitative microdistribution (per 100 μm × 100 μm mesh, step size: 200 μm) in sections of the left and right rat lung lobes ($n = 3$), 24 h after intratracheal administration of a single dose of AEROSIL® P25 TiO_2 nanoparticles (6 mg kg^{-1} body weight) via gavage needle or microsprayer

Lung section		Parameter		Gavage needle	Microsprayer
Left		Content of TiO_2 (ng per mesh)	Mean ± SD	0.034 ± 0.012	0.035 ± 0.0035[d]
			95th percentile	0.21 ± 0.042	0.22 ± 0.023
			Maximum	0.56 ± 0.055	0.74 ± 0.18
		Detection rate (%)*		22 ± 6.4	20 ± 1.0[d]
Right	cranial	Content of TiO_2 (ng per mesh)	Mean ± SD	0.065 ± 0.065	0.050 ± 0.031
			95th percentile	0.32 ± 0.22	0.27 ± 0.13
			Maximum	0.78 ± 0.38	0.74 ± 0.25
		Detection rate (%)*		28 ± 21	25 ± 8.6[d]
	middle	Content of TiO_2 (ng per mesh)	Mean ± SD	0.016 ± 0.0012	0.017 ± 0.0050[d]
			95th percentile	0.13 ± 0.012	0.13 ± 0.031[d]
			Maximum	0.43 ± 0.067	0.67 ± 0.55
		Detection rate (%)*		11 ± 1.4	12 ± 4.4[d,e]
	caudal	Content of TiO_2 (ng per mesh)	Mean ± SD	0.060 ± 0.024	0.098 ± 0.030[a,c]
			95th percentile	0.26 ± 0.056	0.44 ± 0.12[c]
			Maximum	0.59 ± 0.075	0.92 ± 0.28
		Detection rate (%)*		36 ± 14	41 ± 7.0[a,b,c]
	accessory	Content of TiO_2 (ng per mesh)	Mean ± SD	0.052 ± 0.026	0.073 ± 0.023
			95th percentile	0.25 ± 0.079	0.34 ± 0.084
			Maximum	0.65 ± 0.076	0.79 ± 0.21
		Detection rate (%)*		28 ± 13	33 ± 5.8[c]
Entire right		Content of TiO_2 (ng per mesh)	Mean ± SD	0.048 ± 0.021	0.062 ± 0.0093
			95th percentile	0.24 ± 0.067	0.29 ± 0.044
			Maximum	0.61 ± 0.12	0.78 ± 0.30
		Detection rate (%)*		26 ± 10	28 ± 1.2
Entire lung		Content of TiO_2 (ng per mesh)	Mean ± SD	0.043 ± 0.013	0.054 ± 0.0069
			95th percentile	0.23 ± 0.047	0.28 ± 0.040
			Maximum	0.60 ± 0.087	0.77 ± 0.26
		Detection rate (%)*		25 ± 8.6	26 ± 1.2

* The detection rate of TiO_2 is the percentage of the detected analytical points to all analytical points of Ti in each lung section.
[a] There were significant differences from Left lung.
[b] There were significant differences from cranial lobe
[c] There were significant differences from middle lobe.
[d] There were significant differences from caudal lobe.
[e] There were significant differences from accessory lobe.

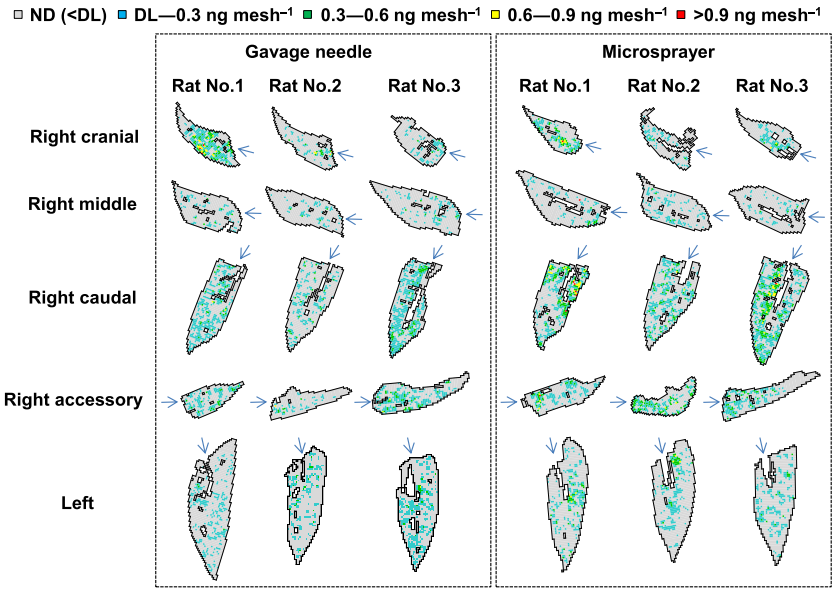

Figure 1. Quantitative maps of the TiO_2 pulmonary microdistribution in sections of the left and right lung lobes of rats ($n = 3$), 24 h after intratracheal administration of a single dose of AEROSIL® P25 TiO_2 NPs (6 mg kg^{-1} body weight) via gavage needle or microsprayer, measured using microbeam X-ray fluorescence microscopy (XGT-7200). The suspension of TiO_2 NPs entered the lung through the lung hilum (indicated by the arrows). Direction of the lung sections represents the direction when the rats were administered the TiO_2 NP suspensions. DL, detection limit; ND, not detectable; NP, nanoparticles.

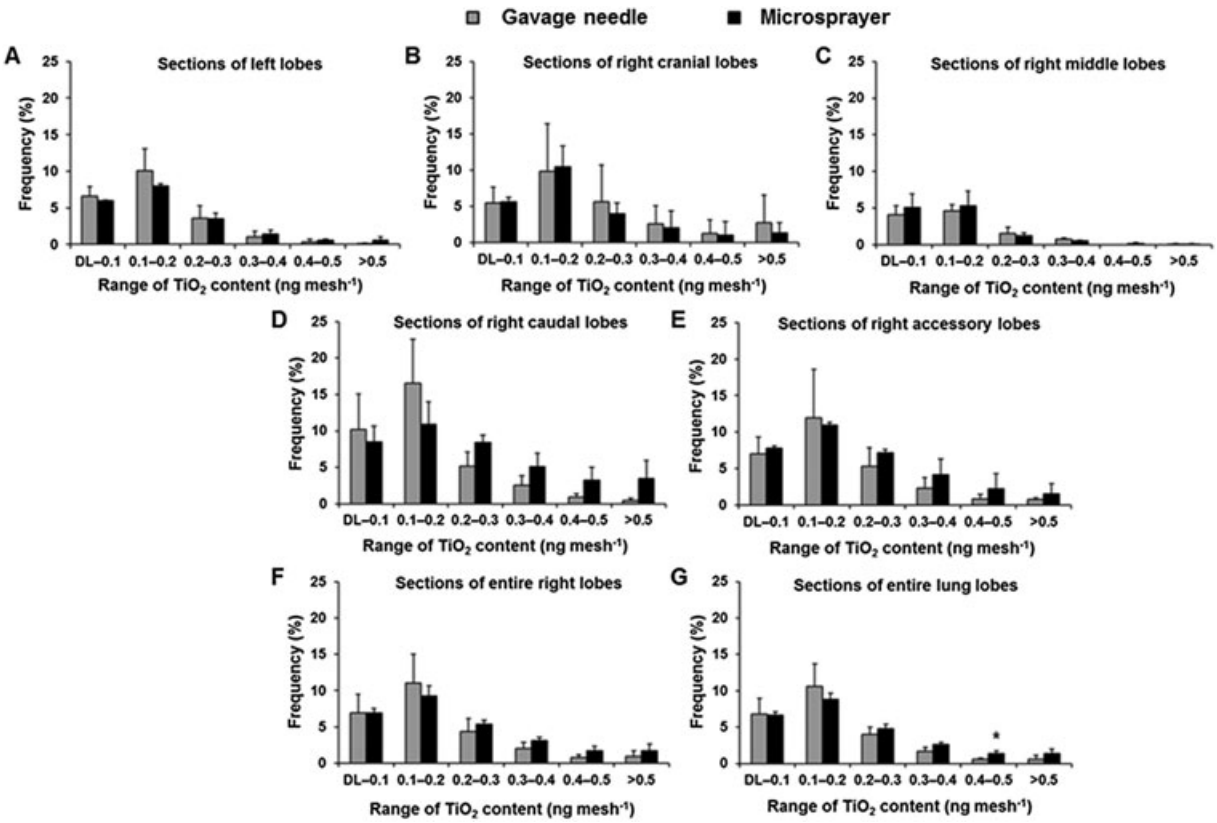

Figure 2. Frequencies of TiO_2 detection in each of the six TiO_2 content ranges for the different rat lung lobe sections ($n = 3$), 24 h after intratracheal administration of a single dose of AEROSIL® P25 TiO_2 nanoparticles (6 mg kg^{-1} body weight) via gavage needle or microsprayer, measured using microbeam X-ray fluorescence microscopy (XGT-7200). (A) Left lobes; (B) right cranial lobes; (C) right middle lobes; (D) right caudal lobes; (E) right accessory lobes; (F) entire right lobes; (G) entire lung lobes. Results are expressed as mean ± SD from three independent animals for each group. *$P < 0.05$ compared with gavage needle. DL, detection limit.

for the right accessory; 43% vs 15% for the entire right; and 30% vs 13% for the entire lungs), except for the right middle lobes (coefficients of variation, $n = 3$, gavage needle vs microsprayer: 7.4% vs 29%).

Discussion

In the current study, we quantitatively evaluated the TiO_2 pulmonary microdistribution in rats administered a single dose of TiO_2 NPs intratracheally (6 mg kg^{-1} body weight) via gavage needle or microsprayer using microbeam XRF microscopy, to compare the differences in the local TiO_2 pulmonary distribution pattern between these two delivery devices.

As described in our previous studies (Zhang *et al*., 2015, 2016), the detected Ti represents the administered TiO_2 because TiO_2 NPs are insoluble particles, and the Ti background is very small.

The data on the pulmonary microdistribution of TiO_2 (Table 1) suggest that similar content (ng $mesh^{-1}$) and frequencies of TiO_2 are deposited in the lung lobes of rats administered TiO_2 NPs via gavage needle and microsprayer. With respect to the detailed pulmonary microdistribution of TiO_2 (Fig. 2), similar frequencies of TiO_2 detection were observed at almost every content range in each lobe, the entire right or the entire lungs, of rats following administration of a single dose of TiO_2 NPs using the different intratracheal delivery devices. Although significantly higher frequencies of TiO_2 detection were observed in the 0.4–0.5 ng $mesh^{-1}$ content range in the entire lungs of rats administered TiO_2 NPs via microsprayer than those administered via gavage needle ($P < 0.05$), this difference is not thought to affect significantly the pulmonary microdistribution of TiO_2 due to the low values in this content range (1.4% for microsprayer vs 0.60% for gavage needle). Moreover, we did not observe significant differences in the 95th percentile and maximum content of TiO_2 between the lung lobe sections of rats administered TiO_2 NPs via gavage needle and microsprayer. These results suggest that there are similar patterns of TiO_2 pulmonary microdistribution when using these two intratracheal delivery devices.

There appears to be less TiO_2 deposition in the right middle lobes, and relatively more in the left and other right lung lobes, irrespective of the intratracheal delivery devices. This observation is in accord with the results of Brain *et al*. (1976) and Leong *et al*. (1998) in which the particles (^{99m}Tc, dye) were intratracheally administered to rats via gavage needle. On the other hand, Brain *et al*. (1976) also reported that via inhalation exposure, the particles (^{99m}Tc) did not show lower deposition in the right middle lobes compared with the left, and right caudal and accessory lung lobes of rats and hamsters. Such evidence suggests that with intratracheal administration, particles have difficulty entering the right middle lung lobes of the experimental animals, which is perhaps due to the experimental design. Potential causes may be modified breathing patterns (anesthesia during intratracheal administration vs normal breathing during inhalation), NP administration form (liquid or liquid aerosols for intratracheal administration vs dry powder aerosols for inhalation), and/or posture of the experimental animals during administration (upright on a slanted board during intratracheal administration vs a prone position during inhalation).

Furthermore, minor variations in the mean content of TiO_2 were observed in rats administered TiO_2 NPs via microsprayer compared with those via gavage needle (e.g., coefficients of variation, $n = 3$, microsprayer vs gavage needle: 13% vs 30% for the entire lungs; 15% vs 43% for the entire right lungs; 9.9% vs 36% for the left lungs). This observation suggests that microsprayers produce a more consistent TiO_2 deposition.

In conclusion, we evaluated quantitatively the pulmonary microdistribution of TiO_2 NPs in rats administered TiO_2 NPs 6 mg kg^{-1} body weight intratracheally via gavage needle or microsprayer using microbeam XRF microscopy. Our results show similar patterns of TiO_2 pulmonary microdistribution in rats administered TiO_2 NPs using these two delivery devices, suggesting that the different physical state of the NP suspension does not markedly alter the local pulmonary distribution of TiO_2. It is possible that administering the NPs via microsprayer leads to the liquid aerosols forming small droplets due to the aerosols colliding with the inner wall of the trachea. The results of this investigation are important for the standardization of intratracheal administration methods.

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Conflict of interest

The authors did not report any conflict of interest.

References

Brain JD, Knudson DE, Sorokin SP, Davis MA. 1976. Pulmonary distribution of particles given by intratracheal instillation or by aerosol inhalation. *Environ. Res.* **11**: 13–33.

Driscoll KE, Costa DL, Hatch G, Henderson R, Oberdorster G, Salem H, Schlesinger RB. 2000. Intratracheal instillation as an exposure technique for the evaluation of respiratory tract toxicity: uses and limitations. *Toxicol. Sci.* **55**: 24–35.

Jacobsen NR, Stoeger T, Brule SVD, Saber AT, Beyerle A, Vietti G, Mortensen A, Szarek J, Budtz HC, Kermanizadeh A, Banerjee A, Ercal N, Vogel U, Wallin H, Moller P. 2015. Acute and subacute pulmonary toxicity and mortality in mice after intratracheal instillation of ZnO nanoparticles in three laboratories. *Food Chem. Toxicol.* **85**: 84–95.

Leong BKJ, Coombs JK, Sabaitis CP, Rop DA, Aaron CS. 1998. Quantitative morphometric analysis of pulmonary deposition of aerosol particles inhaled via intratracheal nebulization, intratracheal instillation or nose-only inhalation in rats. *J. Appl. Toxicol.* **18**: 149–160.

Morimoto Y, Izumi H, Yoshiura Y, Fujishima K, Yatera K, Yamamoto K. 2016. Usefulness of intratracheal instillation studies for estimating nanoparticle-induced pulmonary toxicity. *Int. J. Mol. Sci.* **17**: 165.

Shinohara N, Oshima Y, Kobayashi T, Imatanaka N, Nakai N, Ichinose T, Sasaki T, Zhang G, Fukui H, Gamo M. 2014. Dose-dependent clearance kinetics of intratracheally administered titanium dioxide nanoparticles in rat lung. *Toxicology* **325**: 1–11.

Tada Y, Yano N, Takahashi H, Yuzawa K, Ando H, Kubo Y, Nagasawa A, Inomata A, Ogata A, Nakae D. 2013. Long-term pulmonary responses to quadweekly intermittent intratracheal spray instillations of magnetite (Fe_3O_4) nanoparticles for Fischer 344 rats. *J. Toxicol. Pathol.* **26**: 393–403.

Wang JX, Chen CY, Yu HW, Sun J, Li B, Li YF, Gao YX, He W, Huang YY, Chai ZF, Zhao YL, Deng XY, Sun HF. 2007. Distribution of TiO_2 particles in the olfactory bulb of mice after nasal inhalation using microbeam SRXRF mapping techniques. *J. Radioanal. Nucl. Chem.* **272**: 527–531.

Wang JX, Chen CY, Liu Y, Jiao F, Li W, Lao F, Li YF, Li B, Ge C, Zhou G, Gao Y, Zhao Y, Chai Z. 2008. Potential neurological lesion after nasal instillation of TiO_2 nanoparticles in the anatase and rutile crystal phases. *Toxicol. Lett.* **183**: 72–80.

Yoshiura Y, Izumi H, Oyabu T, Hashiba M, Kambara T, Mizuguchi Y, Lee BW, Okada T, Tomonaga T, Myojo T, Yamamoto K, Kitajima S, Horie M, Kuroda E, Morimoto Y. 2015. Pulmonary toxicity of well-dispersed

titanium dioxide nanoparticles following intratracheal instillation. *J. Nanopart. Res.* **17**: 241.

Zhang G, Shinohara N, Kano H, Senoh H, Suzuki M, Sasaki T, Fukushima S, Gamo M. 2015. Quantitative evaluation of the pulmonary microdistribution of TiO_2 nanoparticles using XRF microscopy after intratracheal administration with a microsprayer in rats. *J. Appl. Toxicol.* **35**: 623–630.

Zhang G, Shinohara N, Kano H, Senoh H, Suzuki M, Sasaki T, Fukushima S, Gamo M. 2016. Quantitative evaluation of local pulmonary distribution of TiO_2 in rats following single or multiple intratracheal administrations of TiO_2 nanoparticles using X-ray fluorescence microscopy. *J. Appl. Toxicol.* **36**(10): 1268–1275.

Supporting information

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